The Use of Thin Layer Chromatography for The Species Identification of Bear Scats

by Harold D. Picton

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FS Contact: L. J. Lyon
Co-op Contact: Harold D. Picton

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Harold D. Picton
Fish and Wildlife Management and Research
Dept. of Biology
Montana State University
Bozeman, Montana
59717

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ABSTRACT

Profiles obtained from known scats by means of thin layer chromatography were used to contruct 2 keys allowing the identification of scats as to species. Factors influencing variation in scats including bile acids and other biological chemicals, age and weathering of scats, method of drying, and the influence of actual and potential food materials were also investigated. Data was obtained from 2011 samples. Two keys for the identification of scats were constructed from 356 profiles from scats of known identity. One of these keys has shown an accuracy of 94% for scats collected during the last half of summer. However, this key also classifies a large proportion of scats as being unknown. The second and newer key appears to be more efficient but has not yet been tested in a comparable manner. Its current accuracy and precision appear to exceed 90%. No single characteristic appears to provide clear species separation. Use of a combination of characters does provide separation. An increase of the size of the data base of known scats, particularly black bears, is highly desirable. Application of the method to areas outside of the Yellowstone and North Continental Divide ecosystems should not be done unless a data base of known scats from the new area is analyzed first.

INTRODUCTION

The conservation of grizzly bears (*Ursus arctos horribilis*) for the future depends upon the effective monitoring of their occurrence, abundance and the ability to gain specific information concerning their biology. The occurrence of sympatric populations of black bears (*Ursus americanus*) has interfered with obtaining this information. The development of a reliable method of identifying the species of bear scats would be a substantial contribution. Bear scats are typically distinguished on the basis of field sign but field sign is often absent when scats are collected. Size or diameter of the scat is not a reliable indicator of species. Calder (1984) indicates that the allometric slope of intestinal diameter is low in mammals suggesting that the larger body size of the grizzly would be reflected in only a small increase in intestine diameter. Scat size is also affected by the nature and quantity of the food consumed.

Major et al (1980) reported that species specific separation of carnivore scats could be obtained by use of thin layer chromatography (TLC) to identify the array of bile acids contained in the scats. Goodwin (1984) in Alaska and Picton (1986) in Montana attempted to apply this technique to the separation of grizzly and black bear scats without success. Plant pigments were a major problem in these studies and considerable revision of the original method was necessary to remove the pigments. Both study groups agreed that TLC assessment of *bile acids* did not provide a reliable means of differentiating scats of the two species. The Alaska study was terminated at this point. Later

evaluation of data from both studies (Picton 1986) suggested that the scats could be differentiated by use of the entire array of spots on the chromatograms regardless of whether or not they were bile acids. This shift away from concentration upon bile acids provided an avenue for progress. The TLC procedure also separates neutral lipids and other lipid soluble unknown constituents along with the bile acids. Exploratory experiments confirmed the possibility of obtaining scat identifications by using TLC methodology. Six of the spots seemed to contain the most species information.

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Table 1. A thin layer chromatography method for the species identification of bear scats.

A. PREPARATION OF STANDARDS

1. Prepare standard solutions of each of the following:

   Cholesterol   Cholic Acid   Lithocholic Acid

   Chenodeoxycholic Acid   Deoxycholic Acid

2. Dissolve 5 mg of each material in 10 ml 1:1 hexane-methanol
3. Use these individual standards to prepare a mixed standard by combining 10 ml aliquots.

B. PREPARATION OF SAMPLES

1. Weigh out a 1 gm sample of the ground (blendorized) air dried fecal material. Use a dust mask and gloves when handling the fecal material.

3. Soak the sample in 125 ml hexane for 24 hours. Then remove the solvent by vacuum filteration. This step serves the function of removing excess plant pigments.
4. Air dry the sample.
5. Soak the sample for 24 hours in 17 ml of 1:1 hexane-methanol.
6. Decant the supernatant, filter through a coarse filter and evaporate.
7. Redissolve the concentrated residue in 0.5 ml 1:1 hexane-methanol.

C. PREPARATION OF THE TLC PLATES

1. Divide the TLC plates into 9 lanes and label each lane.
2. Activate the plate (oven-dried at 120C for 1 hour).
3. Spot 10 ul of the combined standards solution in the 2 side lanes and 20 ul sample in the center lane.

Table 1 continued.

4. Spot the sample solutions in the remaining 6 lanes. Two lanes are used for each sample. One lane should be spotted with 10 ul of the sample and 1 with 20 ul. It may be necessary to reduce these amounts if the pigment concentration is unusually high.

D. DEVELOPING THE PLATES

1. Develop the plates in equilibrated paper lined tanks containing Petcoff's solution (hexane, methylethyl ketone, acetic acid 56:36:8 v/v: (Chavez and Krone 1976).
2. Visualize by dipping the plates in a fresh solution of acetic acid:sulphuric acid: p-anisalehyde 50:1:0.5 v/v.

E. PHOTOGRAPHING THE PLATES

1. Photograph with a 35 mm camera within 15 minutes after removal from the visualizing solution. Kodak Ektachrome ER 135 ASA 100 film may be used. The TLC plate should fill the frame.

   Take 1 exposure (60 sec. f 5.6) using 366nm ultraviolet light.

   Take 1 exposure under normal daylight using the exposure as indicated by the light meter.

F. READING THE PLATE

1. Project the slides on to paper sheet containing a 10 cm grid. The origin spot of the chromatogram should be at 0 and the end of the solvent front should be at 10 cm. The grid should have the r/f values marked off at 0.5 cm intervals. The r/f value is the ratio of the distance travelled by the substance making up a spot to the

Table 1. concluded.

distance travelled by the solvent front. When this plate reading protocol is folloed .5 cm is equal to a r/f value of .05.

2. All squares where a chromatogram spot is located should be checked.
3. Any unusual features of the chromatogram as indicated by the standards should be noted. The center lane standards may indicate slightly higher r/f values than the side lanes.
4. The 20 r/f values and the presence (1) and absence (0) of spots are then used to identify the scat by using the key. Thus 40 data points are available for each plate 20 from the visible light photograph and 20 from the ultra-violet photograph.

It was considered desirable to examine the effects of foods on the TLC profiles. A total of 41 samples from various food plants were analyzed. These were useful in evaluating the removal of plant pigments, in developing the processing method, in testing sample drying methods, determining the influence of weathering and testing the replicability of the results.

Some scats were repetitively analyzed to evaluate the repeatability of the TLC analysis and the storability of scats.

RESULTS

A total of 2011 sample materials were run during the overall study. A number of these materials were run several different times for various purposes. Data was collected from 692 plates,

A summary of the data collected from test standards is presented in table 2. This data was used to generate synthetic profiles which were then compared to the profiles of known grizzly and black bears scats. Bile acids showed a very weak relation to the profiles of either species. Cholic acid and lithocholic acid matched between 28% and 40% of the grizzly bear scats. Lithocholic acid matched 48% of the black bear scats. Florescent patterns typical of bile acids were frequently seen in bear scats but they seem to be of little value in distinguishing species. Cholesterol plus creatinine gave the best match for grizzly bear scats. Cholesterol with estradiol was the best match for black bear scats. The patterns characteristically produced by these materials only partially overlapped the most useful points for distinguishing species.

Table 3. A comparison of TLC profiles of food plants, bears and selected other species.

Species	Conditions	Number of Spots			
		Visible	UV	Florese	%
Affect of Weathering on Ground with Type of Drying					
Blue grass *Poa pratensis*	Fresh				
	Oven dry	10	11	4	36%
	Air dry	11	10	4	40
Blue grass	1 days old after cutting				
	Oven dry	12	11	6	55
	Air dry	12	11	7	64
Blue grass	2 days old after cutting				
	Oven dry	12	9	7	78
	Air dry	12	8	7	88
Blue grass	4 days old after cutting				
	Oven dry	9	7	4	48
	Air dry	9	9	5	38
Blue grass	7 days old after cutting				
	Oven dry	10	10	4	40
	Air dry	10	10	5	50
Blue grass	7 days old after cutting after snow melt				
	Oven dry	7	7	4	31
	Air dry	6	7	4	57

Table 3 continued.

Species	Condition	Visible	UV	Florese	%
Other Plant Species and Type of Drying					
Pine grass	Fresh				
<u>Calamagrostis rubescens</u>					
	Oven dry	10	10	5	50%
Elk sedge	Fresh				
<u>Carex geyeri</u>					
	Oven dry	10	11	9	82
	Air dry	11	10	8	80
Clover					
<u>Trifolium</u> sp.					
	Oven dry	10	10	3	30
	Air dry	9	10	2	20
Evert's Thistle					
<u>Cirsium</u>					
	Oven dry	9	9	3	33
Cow Parsnip					
<u>Heracleum</u>					
	Oven dry	11	13	4	31
Serviceberry					
<u>Amelancier</u>					
	Oven dry	7	9	2	11
	Air dry	7	8	1	13

Table 3 concluded.

Species	Condition	Visible	UV	Florese	%
Mountain Ash berries					
<u>Sorbus scopulina</u>					
	Oven dry	11	10	1	10%
	Air dry	14	9	4	41
Huckleberry					
<u>Vaccinium globulare</u>					
	Oven dry	9	5	3	60
	Air dry	9	10	4	40
Animal Species - All Air Dried					
Grizzly Bear					
<u>Ursus arctos horribilis</u>	n = 22	9	10	4	40
Bear Species (unknown)					
<u>Ursus</u> sp.	n = 25	9	8	3	38
Coyote (Zoo)					
<u>Canis latrans</u>	n = 5	12	9	2	22
Dog					
<u>Canis familiaris</u>	n = 2	14	13	1	4
Mountain Lion (Zoo)					
<u>Felis concolor</u>	n = 2	14	12	0	0

Summary data collected from the samples of known food plants are presented in table 3. The type of drying treatment applied to the sample did not have a significant influence on the TLC results. The amount of weathering that the grass samples received up to one week of leaching did not have a significant influence on the samples. Snow melt leaching may have reduced the number of spots seen in the TLC profiles. Although not entirely obvious from the mean values presented in table 3, bear tended to have slightly fewer spots in their profiles than did the plant materials. The plant materials generally had an abundance of spots at the higher r/f values (r/f = distance of the spot of interest/ distance traveled by the solvent front).

The estimated age of the scats (time since they were dropped to time of collection) was recorded for a set of scats. Correlation coefficients for scat profiles from scats 1, 2 and 3 weeks old and scats 1 day old were 0.7 or greater suggesting that little weathering degradation had occurred.

The scats showed some variation related to the month during which they were collected, however, sample sizes for some months were too small to clearly describe these variations. Comparison of known scats from Yellowstone Park and the North Continental Ecosystem found that September scats from Glacier Park tended to match August scats from Yellowstone better than did August scats from Glacier. This is what might be expected if the TLC scat profiles reflected changes in food habits associated with the annual maturation and drying of the plant communities.

The 42 profiles of 21 known scats were compared to the food

Table 4. A comparison of the profiles of grizzly bear and black bear scats by r/f value.

Visible Light Profiles			Ultra-violet Light Profiles		
	Frequency of Occurrence			Frequency of Occurrence	
r/f	Grizzly Bear	Black Bear	r/f	Grizzly Bear	Black bear
0.05	.99	1.00	.5	.98	1.00
0.10	.28	.41	.10	.25	.49
0.15	.69	.44	.15	.72	.47
0.20	.19	.26	.20	.11	.35
0.25	.12	.15	.25	.09	.15
0.30	.10	.11	.30	.15	.11
0.35	.24	.22	.35	.31	.35
0.40	.20	.30	.40	.27	.39
0.45	.40	.26	.45	.50	.37
0.50	.37	.24	.50	.38	.24
0.55	.45	.30	.55	.51	.26
0.60	.40	.48	.60	.44	.52
0.65	.45	.76	.65	.44	.80
0.70	.55	.76	.70	.58	.85
0.75	.71	.78	.75	.84	.78
0.80	.63	.65	.80	.64	.54
0.85	.51	.44	.85	.45	.41
0.90	.40	.52	.90	.33	.47
0.95	.37	.33	.95	.22	.28
1.0	.39	.28	1.0	.21	.11
N	195	46			

habits analyses for those scats. This permitted comparison of profiles for several levels of grass content, <u>Heraculum</u> <u>lanatum</u>, <u>Equisetum</u>, and moose (<u>Alces</u> <u>alces</u>) in the scats. No clear patterns of difference were seen between these groups of scats, although the moose scats had the lowest correlation values when compared to the vegetation scats. This suggests that a moose or meat diet does affect the TLC profiles of the scats.

Identification keys were contructed from 356 profiles (table 4) obtained from 178 scats of known species. Of this total 14% were black bears from the Yellowstone area and 6% were black bears from the Glacier Park area. Grizzly bear scats represented 80% of the total with 47% from the Greater Yellowstone Ecosystem and 33% from the North Continental Divide Ecosystem.

The first operational key developed (scat key 11.0; Appendix A) emphasized precision or giving an exact identification (grizzly bear or black bear) to each profile in the data base. This key actually consists of 2 keys: 1 for the visible light profiles and 1 for the ultra-violet light profiles. When used against other known as well as unknown samples not included in the data base, it gave ambiguous identifications to 15% of the scats. When this key was subjected to blind tests against groups of scats of known identity a success rate of 66.7% correct identifications was obtained for scats collected from June 1 to July 7th. A 94% success rate for scats collected from July 8 to August 31 (table 5). There were high rates (24-54%) of ambiguous (or no findings) results for both time periods. The differential seasonal success rates may be related to a larger proportion of late summer scats

Table 5. The results of a test of scat key 11.0 against 77 known scats collected from the North Continental Divide and Greater Yellowstone Ecosystems.

Time Period of		Identifcation		
Scat Collection	N	Correct	Incorrect	Ambiguous
June 1 to July 7				
Group I (NCD=9;BB=9)*	17	3 (2BB)	7 (5BB)	7 (2BB)
Per Cent		18%	41%	41%
Group II (NCD=21 GB)	21	15	4	2
Per Cent		71%	19%	10%
Combined	38	47%	29%	24%
Correct identifications were obtained for 66.7% of those for which identifcations were rendered.				
July 8 to August 31				
Group I (NCD=4;BB=1)	15	7 (1BB)	1	7
Per Cent		47%	6%	47%
Group II (NCD=24;1BB)	24	10	0	14 (1BB)
Per Cent		48%	0%	22%
Combined	39	44%	2%	54%
Correct identifications were obtained for 94.4% of those for which identifications were rendered.				

* NCD indicates the number of scats from the North Continental Divide Ecosystem. BB indicates the number of black bear scats; GB indicates the number of grizzly bear scats.

in the key development data base and the low number of black bear scats in the late summer test groups. The ambiguous and erroneous identifications were associated with only 10 per cent of the identification profiles in the key. The identification ratio of the key is 4. This indicates that a new identification profile is needed for each four scat profiles. The learning curve for this key is steepening but is still relatively shallow. This key was used to provide identifications for all of the unknown scats processed.

The high levels of identification failure led to the development of a second key (scat key 12.1; Appendix B). This key had as a goal emphasizing accuracy, rather than precision, and reduction of the rate of ambiguous findings. This key provides precise identifications for many samples but just a probablity estimate is given where there were 3 or less known samples in a key classification category. The probability estimate was calculated from the species of scats falling into the overall category. The probability estimates for scat key 12.1 are presented in table 7. The apparent precision of identification for this key exceeds 90 per cent. The identification ratio for this curve is 8. The key was initially developed using a small data base. As additional scats were added to the data base, the key was modified to handle them. The record of this process summarized as the number of identification profiles added per scat in the enlarged data base provided a "learning curve" for the key. The key has passed through the steep portion of the learning curve suggesting the key will accomodate most scats and scats unknown to it should be less than one in eight. Black bear scats are

not handled as well by the key as are grizzly bear scats. This undoubtedly is a reflection of the number of knowns in the data base. This key now incorporates the known scat data base into its structure and thus test statistics, such as summarized in table 4 for scat key 11.0, are not available.

DISCUSSION

There are several possible sources of variation in scats as well as in the analysis. Scat based variations include: Differing composition among subsamples of the scat; changes in seasonal composition of scats; food habit induced variation including that induced by the consumption of bile acids from the livers of prey animals as well as those associated with the two species; the length of time elapsing between when it was dropped and collected as well as the conditions exposed to during that time. The method of drying and storage and the length of time before final processing could also introduce variation. Variation introduced by the TLC method includes: Variation in the migration rates of the solvent front between plates and between the edges and center within plates; different concentrations of the scat extract (which contains unknown concentrations of the components of interest); minor variations in technique between technicians; inconsistancies in reading slides because of indistinct spots spots spread over several rf units or excessively dark spots. The evidence suggests that the methods and procedures used are robust enough to compensate for these variations and allow sucessful identification.

The ambiguity rate in the precision key 11.0 derives from the fact that it is a "voting" key. As indicated in the methods, each scat is actually run twice on the TLC plates (ie. at two different concentrations). Each of these two TLC lanes rendered an identification from a visible light key and a ultra-violet light key. Thus 4 identifications are actually obtained for each scat. The identification is according to majority vote and if the vote is tied no identification results. The known scats used to test the key gave a high ambiguity rate, much higher than the 15 % rate tabulated for the approximately 1650 unknown scats run.

Key 12.1 is more efficient in the use of information than key 11.0. Key 11.0 uses information from 36 of 40 possible sites while key 12.1 uses information from only 18. Seventy per cent of the sites serve as major information sources for key 11.0. Key 12.1 uses 25% of the sites as primary information sources.

Review of the data indicates that there is no single TLC profile which can serve as a reliable species indicator. The scats appear to vary by characters derived from the species and probably from major differences in food habits. The evidence suggests that there is sufficient difference between the scats of the 2 species to allow reasonably reliable species separation using multiple characteristics. Scats which received an ambiguous determination from key 11.0 should be evaluated with key 12. An increase in the size of the known data base is highly desirable. A larger number of black bear scats is needed. New data bases of known scats would be required to use this method in new areas.

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Appendix A: Table 6. Scat key 11.0.

VISIBLE LIGHT KEY

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A. Sort r/f .65, .7     If = 0 0 --- Category I (gb=.74 bb=.26)
                        If = 0 1 --- Category II (gb=.63 bb=.37)
                        If = 1 0 --- Category III (gb=.48 bb=.52)
                        If = 1 1 --- Category IV (gb=.35 bb=.65)

CATEGORY I   (r/f .65 = 0; .7 = 0)

A. If r/f .2 = 0 --- B
   If r/f .2 = 1 --- Grizzly Bear

  B. If r/f .55 = 0 --- C
     If r/f .55 = 1 --- Grizzly bear

    C. If r/f .15 = 0 --- Black bear
       If r/f .15 = 1 --- D

      D. If r/f .95 = 0 --- E
         If r/f .95 = 1 --- Grizzly bear

        E. If r/f .6 = 0 --- Grizzly bear  (.9 alternate sort)
           If r/f .6 = 1 --- F

          F. If r/f .85 = 0 --- Grizzly bear
             If r/f .85 = 1 --- Black bear

CATEGORY II   (r/f .65 = 0; .7 = 1)

A. Sort r/f .15, .2
    If r/f .15, .2 = 0 0 --- Grizzly bear
    If r/f .15, .2 = 0 1 --- B
    If r/f .15, .2 = 1 0 --- B
    If r/f .15, .2 = 1 1 --- Grizzly bear

  B. If r/f .55 = 0 --- C
     If r/f .55 = 1 --- D

    C. If r/f .85 = 0 --- C1
       If r/f .85 = 1 --- Grizzly bear

      C1. If r/f .6 = 0 --- C2
          If r/f .6 = 1 --- Black bear

       C2  If r/f .9 = 0 --- C3
           If r/f .9 = 1 --- Grizzly bear

        C3 If r/f .5 = 0 --- Black bear
           If r/f .5 = 1 --- C4

         C4 If r/f .35 = 0 --- Black bear
            If r/f .35 = 1 --- Grizzly bear
```

Table 6 continued.

D. If r/f .85 = 0 --- Grizzly bear
If r/f .85 = 1 --- E

E. If r/f 1.0 = 0 --- F
If r/f 1.0 = 1 --- Grizzly bear

F. If r/f .2 = 0 --- Grizzly bear
If r/f .2 = 1 --- Black bear

CATEGORY III (r/f .65 = 1; .7 = 0)

Sort r/f .35, .4, .45, .5

A. If any of these = 1 --- Grizzly bear
If all = 0 --- B

B. If r/f 1.0 = 0 --- C
If r/f 1.0 = 1 --- Grizzly bear

C. If r/f .9 = 0 --- Grizzly bear
If r/f .9 = 1 --- D

D. If r/f .25 = 0 --- E
If r/f .25 = 1 --- Grizzly bear

E. If r/f .75 = 0 --- Black bear
If r/f .75 = 1 --- F

F. If r/f .8 = 0 --- Black bear
If r/f .8 = 1 --- Grizzly bear

CATEGORY IV (r/f .65 = 1; .7 = 1)

A. If r/f .45 = 0 --- B
If r/f .45 = 1 --- E

B. If r/f .3 = 0 --- B1
If r/f .3 = 1 --- B2

B1. If r/f .6 = 0 --- C
If r/f .6 = 1 --- D

B2 If r/f .35 = 0 --- Black bear
If r/f .35 = 1 --- Grizzly bear

C. If r/f .85 = 0 --- C1
If r/f .85 = 1 --- Grizzly bear

C1. If r/f .2 = 0 --- C2
If r/f .2 = 1 --- Grizzly bear

Table 6 continued.

C2. If r/f .75 = 0 --- Grizzly bear
If r/f .75 = 1 --- C3

C3. If r/f .15 = 0 --- Black bear
If r/f .15 = 1 --- C4

C4. If r/f .55 = 0 --- C5
If r/f .55 = 1 --- Black bear

C5. If r/f .8 = 0 --- Grizzly bear
If r/f .8 = 1 --- C5a

C5a. If r/f .1 = 0 --- C5aa
If r/f .1 = 1 --- Black bear

C5aa. If r/f .9 = 0 --- C5ab
If r/f .9 = 1 --- Grizzly bear

C5ab. If r/f .95 = 0 --- Black bear
If r/f .95 = 1 --- Grizzly bear

D. If r/f .2 = 0 --- D1
If r/f .2 = 1 --- Black bear

D1. If r/f .5 = 0 --- D2
If r/f .5 = 1 --- Black bear

D2. If r/f .75 = 0 --- Grizzly bear
If r/f .75 = 1 --- D3

D3. If r/f .35 = 0 --- D4
If r/f .35 = 1 --- Black bear

D4. If r/f .85 = 0 --- D5
If r/f .85 = 1 --- D6

D5. If r/f .1 = 0 --- Black bear
If r/f .1 = 1 --- Grizzly bear

D6. If r/f .8 = 0 --- Black bear
If r/f .8 = 1 --- D7

D7. If r/f .95 = 0 --- Black bear
If r/f .95 = 1 --- Grizzly bear

E. If r/f .8 = 0 --- E1
If r/f .8 = 1 --- EA

E1 If r/f .85 = 0 --- E2
If r/f .85 = 1 --- Grizzly bear

E2 If r/f .4 = 0 --- E3
If r/f .4 = 1 --- Grizzly bear

Table 6 continued.

```
            E3 If r/f 1.0 = 0 --- Black bear
               If r/f 1.0 = 1 --- Grizzly bear


        EA If r/f .2 = 0 --- EB
           If r/f .2 = 1 --- Grizzly bear

           EB  If r/f .15 = 0 --- EC
               If r/f .15 = 1 --- ED

            EC If r/f 1.0 = 0 --- Black bear
               If r/f 1.0 = 1 --- ECa

               ECa If r/f .6 = 0 --- Black bear
                   If r/f .6 = 1 --- Grizzly bear

            ED If r/f .3 = 0 --- EDa
               If r/f .3 = 1 --- Grizzly bear

               EDa If r/f .75 = 0 --- Black bear
                   If r/f .75 = 1 --- EDb

                   EDb If r/f .1 = 0 --- Grizzly bear
                       If r/f .1 = 1 --- Black bear
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ULTRA VIOLET LIGHT KEY

A. Without a spot in r/f 0-.05 --- Grizzly bear
With a spot --- B

B. Without a spot in r/f .05-.1 --- B1
With a spot --- C

B1 With a spot in r/f .95-1.0 --- BA
Without a spot --- B2

BA If r/f .15 = 1 --- Grizzly bear
If r/f .15 = 0 --- Black bear

B2 With a spot in r/f .35-.4 --- B3
Without a spot --- B5

B3 With a spot in r/f .6-.65 --- B4
Without a spot --- Grizzly bear

B4 With a spot in r/f .2-.25 --- Grizzly bear
Without a spot --- BC

BC With a spot in r/f .1-.15 --- BD
Without a spot --- Grizzly bear

BD With a spot in r/f .4- .45 --- Grizzly bear
Without a spot --- BE

BE If r/f .35 = 0 --- Black bear
If r/f .35 = 1 --- BF

BF If r/f .2 = 0 --- Grizzly bear
If r/f .2 = 1 --- Black bear

B5 With a spot in r/f .85-.9 --- B5a
Without a spot --- B9

B5a If r/f .15 = 1 --- B5b
If r/f .15 = 0 --- B5i

B5b If r/f .2 = 1 --- Black bear
If r/f .2 = 0 --- B5c

B5c If r/f .95 = 1 --- Grizzly bear
If r/f .95 = 0 --- B5d

B5d If r/f .3 = 1 --- Grizzly bear
If r/f .3 = 0 --- B5e

B5e If r/f .35 = 1 --- Grizzly bear
If r/f .35 = 0 --- B5f

B5f If r/f .85 = 0 --- Grizzly bear
If r/f .85 = 1 --- B5g

Table 6 continued.

B5g If r/f .65 = 1 --- Black bear
If r/f .65 = 0 --- B5h

B5h If r/f .55 = 1 --- Grizzly bear
If r/f .55 = 0 --- Black Bear

B5i If r/f .2 = 0 --- Grizzly bear
If r/f .2 = 1 --- B5j

B5j If r/f .8 = 1 --- B5k
If r/f .8 = 0 --- Black bear

B5k If r/f .85 = 1 --- Black bear
If r/f .85 = 0 --- Grizzly bear

B9 With a spot in r/f .1-.15 --- BG
Without a spot --- B10

BG With a spot in r/f .15-.2 --- Grizzly bear
Without a spot --- BH

BH If r/f .45 = 1 --- BH1
If r/f .45 = 0 --- BI

BH1 If r/f .55 = 1 --- Grizzly bear
If r/f .55 = 0 --- Black bear

BI If r/f .65 = 1 --- Black bear
If r/f .65 = 0 --- Grizzly bear

B10 With a spot in r/f .6-.65 --- Grizzly bear
Without a spot --- B11

B11 With a spot in r/f .95 --- Grizzly bear
Without a spot --- B12

B12 With a spot in r/f .35 --- Black bear
Without a spot --- B13

B13 If r/f .2 = 1 --- Black bear
If r/f .2 = 0 --- Grizzly bear

C. Without a spot in r/f .65-.7 --- Grizzly bear
With a spot --- D

D. Without a spot in r/f .35-.4 --- E
With a spot --- F

E. Without a spot in r/f .15-.2 --- E1
With a spot --- Grizzly bear

E1 With a spot in r/f .55 --- Grizzly bear
Without a spot --- E1a

Table 6 continued.

```
          E1a If r/f .15 = 1 --- E1b
              If r/f .15 = 0 --- E1c

              E1b If r/f .25 = 1 --- Black bear
                  If r/f .25 = 0 --- E2

               E2 If r/f .2 = 1 --- Black bear
                  If r/f .2 = 0 --- E3

                E3 If r/f .3 = 1 --- Grizzly bear
                   If r/f .3 = 0 --- E4

                 E4 If r/f .65 = 0 --- Grizzly bear
                    If r/f .65 = 1 --- Either black bear or grizzly
                                       bear - go to Co1

              E1c If r/f .9 = 1 --- Grizzly bear
                  If r/f .9 = 0 --- E1d

                  E1d If r/f .35 = 1 --- Black bear
                      If r/f .35 = 0 --- Grizzly bear

      F. With a spot in r/f .25 --- F1a
         Without a spot --- F1

         F1a If r/f .45 = 0 --- Black bear
             If r/f .45 = 1 --- F1b

           F1b If r/f .95 = 0 --- Grizzly bear
               If r/f .95 = 1 --- Black bear

         F1 With a spot in r/f .85 --- F2
            Without a spot --- F4

            F2 If r/f .45 = 1 --- Grizzly bear
               If r/f .45 = 0 --- F3

               F3 If r/f .5 = 1 --- Grizzly bear
                  If r/f .5 = 0 --- Black bear

            F4 If r/f .95 = 1 --- Grizzly bear
               If r/f .95 = 0 --- F5

               F5 If r/f .15 = 1 --- F5a
                  If r/f .15 = 0 --- F6

                  F5a If r/f .3 = 1 --- Grizzly bear
                      If r/f .3 = 0 --- Black bear

                  F6  If r/f .3 = 1 --- Black bear
                      If r/f .3 = 0 --- F6a
```

Table 6 concluded.

```
F6a If r/f .2 = 1 --- Black bear
    If r/f .2 = 0 --- F6b

    F6b If r/f .5 = 1 --- Grizzly bear
        If r/f .5 = 0 --- F6c

        F6c If r/f .45 = 1 --- Black bear
            If r/f .45 = 0 --- Grizzly bear
            (alternate is r/f .6)
```

Appendix B: Table 7. Scat key 12.1 showing the identity probabilities calculated from the data base of known scats.

A. Sort 1. r/f (uv.1+uv.7) (v.7 minus uv.7)

1. 0 0 Sort 2. r/f (v.65+uv.65)
GB probability = .56

0 = Go to sort 3
GB prob.=.69

1 = Grizzly Bear
GB prob.=1.00

2 = Go to sort 4
GB prob.=.26

Sort 3. r/f (v.85+uv.85) (v.5+uv.5)

2 0 = Grizzly Bear
GB prob. = 1.00

0 2 = Grizzly Bear
GB prob. = 1.00

Other = Black bear prob.=.96

Sort 4. r/f (v.85+uv.85) (v.5+uv.5)

1 0 = Grizzly Bear
GB prob. = `1.00

0 2 = Grizzly Bear
GB prob. = 1.00

0 0 = Black Bear
BB prob. = 1.00

2 0 = Black bear prob.=.99

2. 0 1 Sort 2. r/f (v.65+uv.65)
GB probability = .87

0 = Grizzly Bear
GB prob. = 1.00

1 = Grizzly Bear
GB prob. = 1.00

Table 7 continued.

			2 = Black bear prob. = .7
3.	1	-1	Sort 2. r/f (uv.15) GB probability = .39
			0 = Black Bear BB prob. = 1.00
			1 = Grizzly Bear GB prob. = 1.00
4.	1	0	Sort 2. r/f (v.85+uv.85) (v.5+uv.5) GB probability = .53
			0 0 = Sort 3 BB prob. = .69
			Sort 3. r/f (v.9+v.45) (v.9 minus v.4)
			0 0 = Grizzly Bear GB prob. = 1.00
			1 -1 = Grizzly Bear GB prob. = 1.00
			1 1 = Sort 4
			2 0 = Sort 4
			Sort 4. r/f (v.2 minus v.75+v.6)
			0 or 1 = Grizzly Bear GB prob. = 1.00
			Other = Sort 5
			Sort 5. r/f (v.6)
			0 = Grizzly Bear GB prob. = 1.00
			1 = Black Bear BB prob. = .99
(Sort 4 - 2 continued)			0 1 Grizzly Probability = .34

Table 7 concluded.

			Sort 3. r/f (v.9+v.45) (v.9 minus v.45)
			0 0 = Grizzly Bear
			1 -1 = Grizzly Bear
			Other = Black Bear
4-2			Sort 3. r/f (v.9+v.45) (v.9 minus v.45)
			0 0 = Grizzly Bear
			1 1 = either
			2 0 = Grizzly Bear
5.	1	1	= Grizzly Bear
6.	2	-1	Sort 2. r/f (v.65+uv.65)
			0 = Black Bear
			1 = Grizzly Bear
7.	2	0	Sort 2. r/f (v.85+uv.85)
			1 = Grizzly Bear
			Other = Sort 3.
			Sort 3 r/f (v.15 minus v.75+v.6)
			2 = Grizzly Bear
			Other = Sort 4
			Sort 4 r/f (v.35+uv.35)+(v.4+uv.4)
			0 = Grizzly Bear
			4 = Black Bear
			Other = p .85 Black Bear